

CLAIMS

5 1. Anti-aurora-A monoclonal antibody specifically recognizing human and murine aurora-A kinase, and having the following properties:

- * it can be fixed on the membranes containing the human or murine aurora-A protein,
- * it allows detection, and, if appropriate, purification, of the human and murine aurora-A protein by immunoprecipitation,
- 10 * it allows the staining of biological tissues where the aurora-A protein is secreted, and
- * it does not inhibit the enzymatic activity of the human and murine aurora-A protein,

15 said monoclonal antibody being as obtained by:

– five injections spread over fifteen days to mice of recombinant aurora-A protein kinase produced by *E. coli* bacteria transformed with a bacterial expression vector in the genome of which the human cDNA coding for aurora-A has been inserted, sacrificing said mice, and fusion between cells of the spleen of these mice and hamster cells immortalized in culture in order to obtain hybridomas,

20 – screening of the hybridomas producing an antibody capable of immunoprecipitating the recombinant protein used for the immunization of the mice during the preceding stage, and recovery of the positive hybridomas after this first screening,

25 – screening of the hybridomas recovered in the preceding stage, producing an antibody capable of immunoprecipitating the endogenous aurora-A protein from an extract of human HeLa cells in culture, and recovery of the positive hybridomas after this second screening,

30 – screening of the hybridomas recovered in the preceding stage, producing an antibody capable of recognizing in indirect immunofluorescence the centrosomes and the poles of the mitotic spindle of human cells in culture, and recovery of the positive hybridomas after this third screening,

– screening of the hybridomas recovered in the preceding stage, producing an antibody capable of immunoprecipitating the endogenous aurora-A protein of mice

from an extract of murine cells in culture, and recovery of the positive hybridomas after this fourth screening,

- screening of the hybridomas recovered in the preceding stage, producing an antibody capable of recognizing in indirect immunofluorescence the centrosomes and the poles of the mitotic spindle of murine cells in culture,
- recovery and purification by cloning of a positive hybridoma after the preceding screening stage, and production of a monoclonal antibody possessing all of the properties defined above.

10 2. Monoclonal antibody according to claim 1, also called 35C1 antibody, as secreted by the hybridoma deposited at the Collection Nationale de Cultures de Microorganismes (CNCM) of the Institut Pasteur under the number I-3050.

15 3. Use of a monoclonal antibody as defined in claim 1 or 2, for the implementation of an *in vitro* diagnostic or prognostic method for cancers in humans or animals.

20 4. Use of a monoclonal antibody according to claim 3, for the implementation of an *in vitro* diagnostic or prognostic method for solid tumours such as breast cancers, stomach cancers and colorectal cancers.

5. Use according to claim 3 or 4, in combination with a cell proliferation marker, such as a marker of the PCNA protein.

25 6. An *in vitro* diagnostic or prognostic method for cancers as defined in claim 4, in humans or animals, characterized in that it comprises:

— placing a monoclonal antibody according to claim 1 or 2, in the presence of a biological sample taken from an individual, said antibody if appropriate being fixed on a solid support,

30 — the detection, and if appropriate the quantitation, of the aurora-A protein which may be present in the biological sample using marked reagents, in particular marked antibodies, recognizing either the monoclonal antibody linked to said aurora-A protein, or the aurora-A protein linked to said monoclonal antibody in the complexes formed during the preceding stage between the monoclonal antibody and the aurora-A protein

which may be present in the biological sample, this, if necessary, after appropriate rinsing of the solid support.

5 7. Method according to claim 6, characterized in that the determination of a quantity of aurora-A protein lower than or greater than the normal physiological values in the biological sample, shows respectively a good or a poor prognosis for the diagnosed cancer.

10 8. Kit for the implementation of a diagnostic method according to claim 6 or 7, characterized in that it comprises:

- an anti-aurora-A monoclonal antibody according to claim 1 or 2,
- if appropriate, a cell proliferation marker, such as a marker of the PCNA protein, in particular an anti-PCNA antibody.

15 9. Use of an antibody defined in claim 1 or 2, for the preparation of medicaments intended for the treatment of cancers, such as breast cancers, colorectal cancers and stomach cancers.

20 10. Pharmaceutical composition containing an antibody according to claim 1 or 2, in combination with a pharmaceutically acceptable vector.

25 11. Use of an anti-aurora-A monoclonal antibody according to claim 1 or 2, for the implementation of a method for screening inhibitors of aurora-A kinase in which the lowering of the activity of this kinase is measured using said antibody.

12. Method for screening inhibitors of aurora-A kinase characterized in that it comprises the following stages:

- the treatment of cells, such as lines derived from different cancers, with the inhibitor tested,
- immunoprecipitation of the aurora-A protein kinase using an antibody according to claim 1 or 2, and measurement of the kinase activity.

13. Method for the preparation of an anti-aurora-A monoclonal antibody according to claim 1 or 2, characterized in that it comprises the following stages:

5 – five injections spread over fifteen days to mice of recombinant aurora-A protein kinase produced by *E. coli* bacteria transformed with a bacterial expression vector in the genome of which the human cDNA coding for aurora-A has been inserted, sacrificing said mice, and fusion between cells of the spleen of these mice and hamster cells immortalized in culture in order to obtain hybridomas,

10 – screening of the hybridomas producing an antibody capable of immunoprecipitating the recombinant protein used for the immunization of the mice during the preceding stage, and recovery of the positive hybridomas after this first screening,

15 – screening of the hybridomas recovered in the preceding stage, producing an antibody capable of immunoprecipitating the endogenous aurora-A protein from an extract of human HeLa cells in culture, and recovery of the positive hybridomas after this second screening,

20 – screening of the hybridomas recovered in the preceding stage, producing an antibody capable of recognizing in indirect immunofluorescence the centrosomes and the poles of the mitotic spindle of human cells in culture, and recovery of the positive hybridomas after this third screening,

25 – screening of the hybridomas recovered in the preceding stage, producing an antibody capable of immunoprecipitating the endogenous aurora-A protein of mice from an extract of murine cells in culture, and recovery of the positive hybridomas after this fourth screening,

 – screening of the hybridomas recovered in the preceding stage, producing an antibody capable of recognizing in indirect immunofluorescence the centrosomes and the poles of the mitotic spindle of murine cells in culture,

 – recovery and purification by cloning of a positive hybridoma after the preceding screening stage, and production of a monoclonal antibody possessing all of the properties defined in claim 1.